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IN VITRO CALLOGENESIS IN BRYONOPSIS LACINIOSA (L). NAUD

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ABSTRACT

Bryonopsis laciniosa (L.) Naud is an endangered and valuable medicinal plant belongs to the family Cucurbitaceae. It is commonly known as lollipop climber and is globally distributed in the paleotropics. *Bryonopsis* plants are used to treat various types of diseases. Different explants like leaf, cotyledon, nodal, stem and branch segments were tested on MS medium supplemented with different concentrations of 2, 4 – D, BAP, IBA, NAA, TDZ and L – glutamic acid. Excess of callus growth occurred in nodal explants on MS medium fortified with 2.0 mg/l 2, 4 – D and 5.0 mg/l BAP. It was observed that among the explants tested nodal, cotyledon and stem explants were key material for excellent callus proliferation and growth.

KEYWORDS

Callogenesis, Nodal, 2, 4 – Dichlorophenoxy Acetic Acid, 6 – Benzyl Amino Purine, *Bryonopsis laciniosa.*

INTRODUCTION

Bryonopsis laciniosa (L.) Naud is an endangered and valuable medicinal cucurbit which is attributed with pharmacological properties. It is commonly known as lollipop climber and is globally distributed in the paleotropics. *Bryonopsis* plants are annual scaberulous herbs used to treat adenopathy, asthma, head-ache, paralysis, phthisis, tuberculosis etc.

The bioactive molecule goniothalamin isolated from this plant showed potent cytotoxicity, weak antibacterial and significant antifungal activity against a wide range of gram positive and gram negative bacteria and fungi (Mosaddik, M.A. and M. Ekramul Haque, 2003). It was also highly effective against the larvae of the mosquitos. (Kabir *et al* 2003). The Cytotoxic activity of (S) goniothalamin and analogues isolated were evaluated against eight human cancer cells (Fatima *et al*., 2006).

Plant tissue culture technology is an alternative method for conserving germplasm in a vegetative state to produce a large number of plants on demand (Arora and Bhojwani, 1989)

Callus is an unorganized and amorphous mass of loosely arranged undifferentiated cells develops from parent tissue due to proliferation of cells in *in vitro* conditions.

Application of plant tissue culture methods in crop improvement depends upon the induction of viable callus cultures and maintenance of *in vitro* conditions (vasil *et al*, 1979).

Callus cultures can easily be established on an appropriate medium for multicellular explants of living tissues originating from different parts of plant such as, shoot tips, stem, leaf, root, nodal, cotyledon, inflorescence etc.

All the multicellular plants are capable and potential sources of explants for the induction of callus (Yeoman and Macleod, 1977).

Different explants respond differently for the callus induction and also depends on different concentrations of hormones, nutritions and factors(Trigiano and Gray,2000). Induction of callus from highly organized structures such as leaves, shoots, cotyledons, hypocotyls is called dedifferentiation and production of organized plant parts from callus is called regeneration. Dedifferentiation and regeneration, these two components of tissue culture play an important role in crop improvement, disease elimination, germ plasm preservation, rapid and mass production of plants.

The growth and development of the tissues from different explants used depend upon the composition of the media used (Attchison, Macleod and Yeoman, 1977; Ainsley *et al*, 2000).

Callogenic initiation implies an initial stage of differentiation from the parental tissue thus the determination of the initial tissue is a fundamental factor in order to achieve the desired response (Bandyopadhyay *et al*, 1999).

In general, the cotyledon and stem explants seem to be best suited for callus induction. There are some reports on callogenesis in plants such as *Hemidesmus* (Sarasan *et al*, 1994), *Amorphophallus* (Nyman *et al*, 1987), *Ceratonia siliqua* (Martins – Loucao and Rodriguez – Barueco, 1981), *Cassava* (Eskes *et al*, 1974; Prabhudesai and Narayana Swamy, 1975; Parke, 1978; Rey and Fernandez, 1980), *Pisum sativum* (Malomberg, 1979); *Crotalaria medicagenia* (Raj Bhansali *et al*, 1978),*Phaseolus vulgaris* (Crocomo *et al*, 1976) and *Dioscorea* (Chaturvedi, 1975; Sinha and chaturvedi, 1979 and Mascarenhas *et al*, 1976)

MATERIALS AND METHODS

The plants and seeds of *Bryonopsis laciniosa* (L) Naud were collected from local forest area of Khammam and Warangal Districts. A.P. and were grown in University campus garden.

The healthy and young explants stem, nodal,branch,leaf and cotyledon were selected and washed thoroughly in running tap water for 20 minutes, then the explants were soaked in 70% alcohol for 5 minutes and surface sterilized with 1.0% mercuric chloride solution and left on a shaker for 6 – 7 minutes and then the explants were rinsed in sterile distilled water for 3 – 4 times to discard the traces of mercuric chloride. The explants were cultured on MS Medium supplemented with different concentrations of auxins and cytokinins. The PH of the medium was adjusted to 5.7 to 5.8 using 0.1 NaoH or Nacl before autoclaving. 15 ml of the medium were dispensed in each culture tube and tightly closed with non - absorbent cotton bunks wrapped with gauze cloth and autoclaved at 15 Psi for 20 minutes. All the cultures were maintained at 16 hr photoperiod followed by 8 hrs darkness with 400 lux light.
The growth measurements of calli were recorded as a function of increase in the fresh and dry weights after a period of 30 days. For determination of callus fresh weight, the moisture was removed by gently blotting on a filter paper and transferred to a pre – weighed aluminium foil and the dry weight was determined after drying the tissue at a temperature of 60^0c in an oven. The average wet (fresh) and dry weight was used to compare the growth response at particular conditions.

RESULTS AND DISCUSSIONS

Callus inducing ability of different explants like leaf, cotyledon,nodal, stem and branch segments of *Bryonopsis laciniosa* were tested. Among all the explants tested nodal, cotyledon and stem explants were responded well for the induction of callus.

Nodal explant :
When nodal explant was inoculated on MS medium supplemented with 2.0 mg/l 2,4 – D and 5.0 mg/l BAP initiated callus. (Plate 1, fig 1) Profused growth of friable callus was produced in long term callus cultures on same composition of medium (Plate 1, fig 2). Rooting was observed in long term callus cultures when cultured on MS medium fortified with 2.0 mg/l 2, 4 – D and 1.0 mg/l IBA (plate 1, Fig 3) and turned friable callus into dark brown callus in long term cultures on the above same medium (plate 1, Fig 4). Callus response from nodal explant comparatively encouraging.

Plate 1
Induction of callogenesis from nodal cultures of Bryonopsis laciniosa (L.) Naud
Figure 1
Initiation of callus from nodal explant on MS + 2.0 mg/l 2,4 – D + 0.5 mg/l BAP.

Figure 2
Profused growth friable callus in long term callus cultures

Figure 3
Rooting of long term callus cultures on MS + 2.0 mg/l 2, 4 – D + 1.0 mg/l IBA

Figure 4
Turning of friable callus into dark brown callus in long term callus cultures.

***Cotyledon explant*:**
The cotyledons were inoculated on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA initiated callus (plate 2, Fig 1). On the same medium after sub – culture initiated rhizogenesis (plate 2, Fig 2). The explants inoculated on MS medium containing 1.0 mg/l BAP and 2.0 mg/l 2, 4 – D formed green callus (plate 2, Fig 3). MS medium supplemented with 2.0 mg/l NAA and 0.5 mg/l TDZ observed 60% green shoot buds from callus (plate 2, Fig 4).

Plate 2
Induction of callus from cotyledon of *Bryonopsis laciniosa* (L.) Naud

Figure 1
Initiation of callus from cotyledon explant on MS + 1.0 mg/l BAP + 0.5 mg/l NAA.

Figure 2
Initiation of rhizogenesis after sub culture on the same medium as above

Figure 3
Greening of callus on MS + 1.0 mg/l BAP + 2.0 mg/l 2, 4 – D.

Figure 4
Green shoot buds from callus on MS + 2.0 mg/l NAA + 0.5 mg/l TDZ

Stem explant:
The stem explants were inoculated on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA initiated callus (Plate – 3, Fig 1) medium containing 1.0 mg/l BAP and 0.5 mg/l 2,4 – D initiated globular callus (Plate 3, Fig 2) and the percentage of frequency of growth response was 40%. When the concentration of 2, 4 – D (3.0 mg/l) increased to the above same medium green callus was formed (Plate 3, Fig 3). Medium containing 2.0 mg/l NAA and 0.5 mg/l TDZ regeneration was observed from callus derived from stem explants (Plate 3, Fig 4).

Callus was initiated from stem explant when cultured on MS medium containing 1.0 mg/l BAP and 1.0 mg/l IAA (plate 3, fig 5). A globular callus was formed when the explant was inoculated on MS medium with 2.0 mg/l Kn and 1.0 mg/l TDZ (plate 3, fig 6). Addition of L – glutamic acid (amino acid) to medium containing 1.0 mg/l BAP and 1.0 mg/l TDZ observed greening and regeneration of callus (plate 3, fig 7). When 2.0 mg/l NAA and 0.5 mg/l TDZ added to MS medium rhizogenesis was observed from callus derived explants (plate 3, fig 8).

Plate 3
Induction of callus and regeneration from stem explant cultures of *Bryonopsis laciniosa*
Figure 1
Initiation of callus from stem explant on MS + 1.0 mg/l BAP + 0.5 mg/l NAA.

Figure 2
Initiation of globular callus on MS + 1.0 mg/l BAP + 0.5 mg/l 2,4 – D.

Figure 3
Greening of callus on MS + 1.0 mg/l BAP + 3.0 mg/l 2, 4 – D.

Figure 4
Regeneration from callus derived from stem explant on MS + 2.0 mg/l NAA + 0.5 mg/l TDZ.

Figure 5
Initiation of callus from stem explant on MS + 1.0 mg/l BAP + 1.0 mg/l IAA

Figure 6
Initiation of globular callus on MS + 2.0 mg/l Kn + 1.0 mg/l TDZ

Figure 7
Greening and regeneration of callus on MS + 1.0 mg/l BAP + 1.0 mg/l TDZ + 1.0 mg/l L – glutamic acid.

Figure 8
Rhizogenesis from callus derived explant on MS + 2.0 mg/l NAA + 0.5 mg/l TDZ

In the present investigation the effect of various auxins and cytokinins on different explants had evoked different morphogenetic responses(Table 1). This is in accordance with Rossi Hossani and Zyrd, 1995, different growth regulators affects callus texture and morphology.

TABLE - 1

EFFECT OF VARIOUS GROWTH REGULATORS ON MORPHOGENETIC RESPONSE FROM COTYLEDON, STEM AND NODAL EXPLANTS OF *BRYONOPSIS LACINIOSA* (L.) NAUD.

Growth regulators (mg/l)	Cotyledon		Stem		Nodal	
	% frequency of growth response	Morphogenetic response	% frequency of growth response	Morphogenetic response	% frequency of growth response	Morphogenetic response
2.0 2,4-D + 5.0 BAP	42	Callus	40	Callus	44	Friable callus
2.0 2,4-D + 1.0 IBA	35	Friable callus	42	Compact callus	40	Rooting
2.0 NAA + 0.5 TDZ	50	Green shoot buds	45	Greening callus	50	Compact callus
2.0 NAA + 0.5 TDZ	60	Green shoot buds	50	Rhizogenesis	45	Globular callus
2.0 Kn + 1.0 TDZ	50	Brown compact hard callus	60	Globular callus	55	Green callus
1.0 BAP + 1.0 TDZ + L-Glutamic acid	40	Green patches on callus	35	Greening of callus	45	Friable callus
1.0 BAP + 0.5 NAA	25	Initiation of rhizogenesis	40	Initiation of callus	30	Callus
1.0 BAP + 1.0 2,4-D	30	Compact callus	20	Initiation of callus	30	Compact callus
1.0 BAP + 1.0 TDZ	25	Hard compact callus	30	Direct regeneration	25	Compact callus

The present study deals with the efficiency of callusing on MS media fortified with growth regulators from different explants of *Bryonopsis* such as cotyledon, stem and nodal. The cotyledon and nodal cultures on medium supplemented with 2,4 – D and BAP could form greening of callus at the cut ends of explants but the percent of growth response was less. 2,4 – D is a potent auxin stimulating callogenesis (Trifonova *et al.,*2001; Da Silva *et al*.,2005)but totally suppresses root and shoot bud formation (Murashige, 1974).In *Bryonopsis*

2,4-D promoted the callus formation,similar observations were made in *Arachis hypogea* (Narasimhulu and Reddy, 1983), *Petunia* (Rao *et al*, 1973) *Capsicum* (Gunay and Rao, 1978, Philips and Hubstenberger, 1985). *Mathiola incana* (Gautham *et* al, 1983)

In the present study callus was formed from various explants, which were cultured on MS medium fortified with the combinations of 1.0 mg/l BAP and 0.5 mg/l NAA. After sub – culture on the same medium initiated rhizongenesis. It was observed that direct regeneration took place on MS medium supplemented with BAP and TDZ. Abundant callus was formed on MS medium supplemented with 2, 4 - D in combination with NAA from most of the explants studied especially stem explant. Raj Bhansali *et al*, (1978) reported the same observations in *Crotalaria burnia* and Bingham et al.,(1975) in *Medicago sativa*. Excess of callus growth occurred in nodal explant on MS medium supplemented with 2.0 mg/l 2,4 – D and 5.0 mg/l BAP. Similar results were observed in *Triticum aestivum* var. Lu – 26 S (Shah *et al*, (2003);*Elaeocarpus robustus* Roxb(Rahman et al., 2004b); *Canavalia brasiliensis* (Da Silva *et al.,*2005); *Galdiolus hybridus* Hort. (Aftab *et al*, (2008).

In general high concentration of auxin to low concentration of cytokinin promotes callus formation(Flick *et al.*,1983;Grattapaglia and Machado 1990). Where as in the present study best callusing response was observed with low concentration of auxin to cytokinin

2. 4 – D were most suitable combinations for the callus formation to the NAA and other auxins (Table 2). Similar results were also reported by other workers (Templeton – Somers, 1986;Templeton – Somers and Collins, 1985, Unnikrishnan and Mukerjee, 1991). Kumar *et al*, (1983) reported exuberant callus growth in *Arbidopsis* on basal medium supplemented with 2.0 mg/l 2, 4 – D + 0.5 mg/l Kn. Abundant callus was initiated in scented *Pelargonium* with 2.4 – D in combination with Kn (Jacqueline and Chalwood, 1986).

TABLE - 2

EFFECT OF DIFFERENT AUXINS (0.5 - 2.0 mg/l) ON CAULOGENESIS FROM DIFFERENT EXPLANTS OF *BRYONOPSIS LACINIOSA* (L.) NAUD

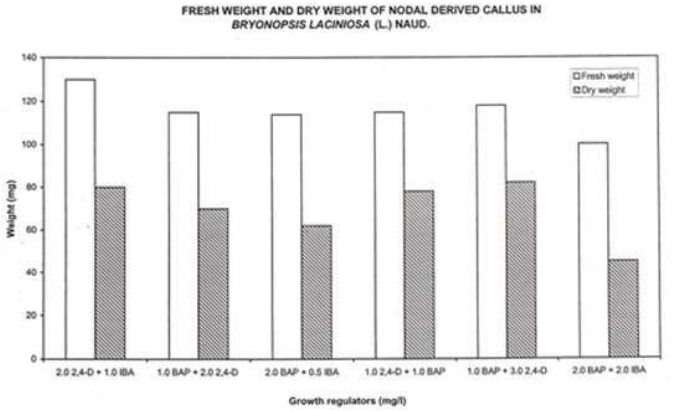
Explant	2,4-D		NAA		TDZ		BAP	
	% of response	Nature of response	% of response	Nature of response	% of response	Nature of response	% of response	Nature of response
Nodal	62	***+	45	++	40	**	20	++
Cotyledon	70	***	55	+++	35	+*	15	++
Stem	55	**	40	*+	42	++	25	**

* Callus
*** Profuse callus
** Moderate callus
* Scanty callus
\+ Rooting
+++ Extensive rooting
++ moderate rooting
+ Scanty rooting

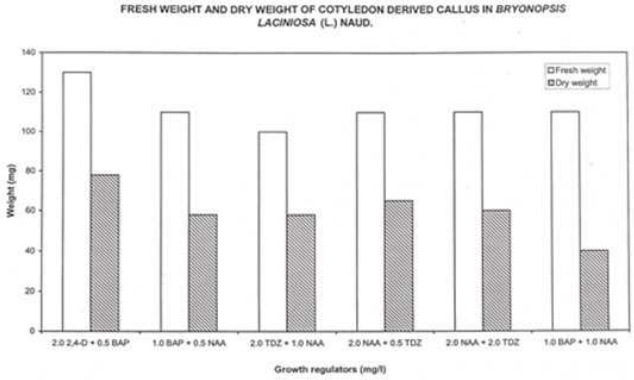
Different concentrations of auxin and cytokinin combinations yielded maximum callogenesis response(Anzidei *et al.*,2000;Castillo *et al.,*2000).

Auxin and cytokinin (BAP, IBA, TDZ, NAA and 2,4 – D) could yield highest amount of callus from nodal, cotyledon and stem explant cultures in fresh and dry weights (graph 1, 2 and 3)

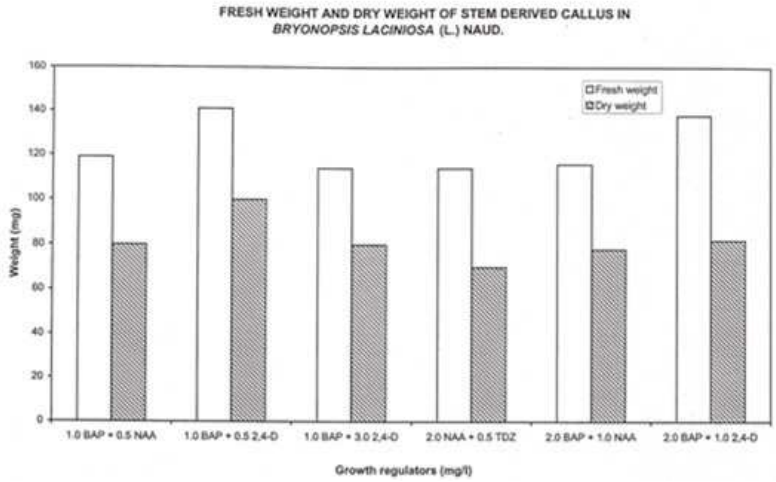
GRAPH-1



GRAPH-2



GRAPH-3



During the present investigation the callus was mostly of friable nature. This might be because 2,4 – D induces friable callus formation (Tenimoto and Harada, 1980). The late development of compact callus might be due to partial depletion of exogenous 2,4 – D and balance between external and internal growth factors (Kumar *et al*, 1983; Kumar 1988). Addition of NAA in combination with BAP favoured root and shoot regeneration in callus from most of the explants studied. Growth of callus was reduced with higher concentrations of NAA. 2, 4 - D and NAA better suited than IAA for callus initiation. BAP in combination with Kn or BAP alone did not respond rapid growth of callus. Acoording to Steinhert (1981) BAP and Kn have an additive effect on growth of callus.

All the explants sources tested in this study developed callus to varying degrees on various growth regulators combinations evaluated (Table 3, 4 & 5). Among all the explants tested cotyledon, stem and nodal explants were key material for excellent callus proliferation and growth. Frequent sub – cultures were necessary to make the callus viabale for further investigation.

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TABLE - 3

EFFECT OF DIFFERENT GROWTH REGULATORS ON CALLUS INDUCTION FROM STEM EXPLANTS OF *BRYONOPSIS LACINIOSA* (L.) NAUD

Growth regulators in mg/l				Amount of callus produced	% of response
BAP	NAA	2,4-D	TDZ		
0.5	0.5	--	--	0	0
1.0	0.5	--	--	++	25
1.5	0.5	--	--	+	10
2.0	0.5	--	--	0	0
0.5	--	0.5	--	+	25
1.0	--	0.5	--	+++	40
1.5	--	0.5	--	++	35
2.0	--	0.5	--	0	0
--	0.5	--	0.5	0	0
--	1.0	--	0.5	+	20
--	1.5	--	0.5	++	35
--	2.0	--	0.5	+++	40

No of replicates 10, growth period 50; O = No response; + Slight callus formation
++ : Moderate callus formation; +++ : High callus formation; ++++ : Intense callus formation

TABLE - 4

EFFECT OF DIFFERENT GROWTH REGULATORS ON CALLUS INDUCTION FROM NODAL EXPLANTS OF *BRYONOPSIS LACINIOSA* (L.) NAUD

Growth regulators in mg/l			Amount of callus produced	% of response
2,4-D	BAP	IBA		
2.0	0.5	--	0	0
2.0	1.0	--	+	12
2.0	2.0	--	++	24
2.0	3.0	--	++	28
2.0	4.0	--	+++	32
2.0	5.0	--	++++	48
2.0	6.0	--	++	28
2.0	--	0.5	++	26
2.0	--	1.0	+++	36
2.0	--	1.5	++	25
2.0	--	2.0	++	27
2.0	--	2.5	+++	40
2.0	--	3.0	++	28

No of replicates 10, growth period 50; O = No response; + Slight callus formation
++ : Moderate callus formation; +++ : High callus formation; ++++ : Intense callus formation

TABLE - 5

EFFECT OF DIFFERENT GROWTH REGULATORS ON CALLUS INDUCTION FROM COTYLEDON EXPLANTS OF *BRYONOPSIS LACINIOSA* (L.) NAUD

Growth regulators in mg/l				Amount of callus produced	% of response
BAP	NAA	2,4-D	TDZ		
0.5	0.5	--	--	0	0
1.0	0.5	--	--	+	10
1.5	0.5	--	--	+++	30
2.0	0.5	--	--	++	20
0.5	--	2.0	--	+	10
1.0	--	2.0	--	+++	25
1.5	--	2.0	--	++	15
2.0	--	2.0	--	+	10
--	0.5	--	0.5	0	0
--	1.0	--	0.5	+	10
--	1.5	--	0.5	++	20
--	2.0	--	0.5	+++	30

No of replicates 10, growth period 50; O = No response; + Slight callus formation
++ : Moderate callus formation; +++ : High callus formation; ++++ : Intense callus formation

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